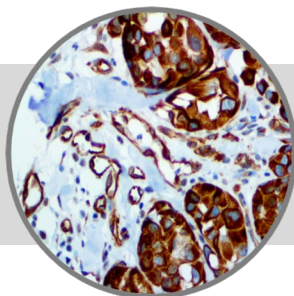


Nestin, RMab

Clone: EP287

Rabbit Monoclonal


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Inset: IHC of Nestin on a FFPE Melanoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The Nestin antibody, clone EP286, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

Cyclic peptide corresponding to C-terminus of human Nestin.

Summary and Explanation

Nestin is a type VI intermediate filament protein; they are expressed mostly in nerve cells where they are implicated in the radial growth of the axon. Nestin is expressed in dividing cells during the early stages of development in the Central Nervous System (CNS), Peripheral Nervous System (PNS) and in myogenic and other tissues. Nestin is expressed by many types of cells during development, although its expression is usually transient and does not persist into adulthood. Nestin is however expressed in the neuronal precursor cells of the subgranular zone in adult organisms. Its expression is also reinduced in the adult during pathological situations, such as the formation of the glial scar after CNS injury and during regeneration of injured muscle tissue.

It has been reported that Nestin expression is significantly increased in melanoma and correlated with more advanced stages of the disease. It has also been reported in tumors of the CNS, including astrocytoma, ependymoma, oligodendroglioma, glioblastoma, and primitive neuroectodermal tumors, as well as in carcinomas such as prostatic adenocarcinoma, pancreatic ductal carcinoma, thyroid carcinoma, and in mesenchymal tumors. In breast carcinoma subtypes, Nestin is highly expressed in basal breast cancer but not in the HER2 subtype or luminal epithelial phenotype. In normal skin, Nestin is expressed in endothelial cells and the bulge area of hair follicles.

Antibody Type	Rabbit Monoclonal	Clone	EP287
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Kidney, Breast, Adrenal, Myometrium, Liver Carcinoma
Species Reactivity		Human, Mouse	

Presentation

Nestin is a purified immunoglobulin fraction of rabbit antiserum that is filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

<i>Catalog No.</i>	<i>Antibody Type</i>	<i>Dilution</i>	<i>Volume/Qty</i>
BSB 2000	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2001	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2002	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2003	Concentrated	1:50 - 1:200	0.1 mL
BSB 2004	Concentrated	1:50 - 1:200	0.5 mL
BSB 2005	Concentrated	1:50 - 1:200	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB 2006	5 slides

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN₃) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.









Product Limitations

Normal Tissues	
Positive (+)	
Endothelial Cells	Pituitary
Hair Follicles	Myoepithelial Cells, Breast
Melanocytes	Smooth, Skeletal Muscle
Negative (-)	
Brain	Adrenal Cortex
Abnormal Tissues	
Positive (+)	
Melanoma 15/15	GIST 10/10
Kaposi Sarcoma 2/2	Breast Carcinoma 6/45
Negative (-)	
Colon Carcinoma 0/20	Hepatocellular Carcinoma 0/5
Lung Carcinoma 0/5	Papillary Thyroid Carcinoma 0/5

References

1. Michalczyk K, et al. Histol Histopathol. 2005 April; 20(2):665-71
2. "About intermediate filaments." Cytoskeleton, Inc.
3. Brychtova S, et al. J Cutan Pathol. 2007; 34:370-75
4. Maho K, et al. Journal of Dermatology. 2010; 37:505-11
5. Toshiyuki I, et al. World J Gastroenterol. 2011; 17:409-18
6. Li H, et al. Cancer Res. 2007; 67:501-10
7. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller		Catalog Number Référence du catalogue Bestellnummer
	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum		Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis		Lot Number Code du lot Chargenbezeichnung

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